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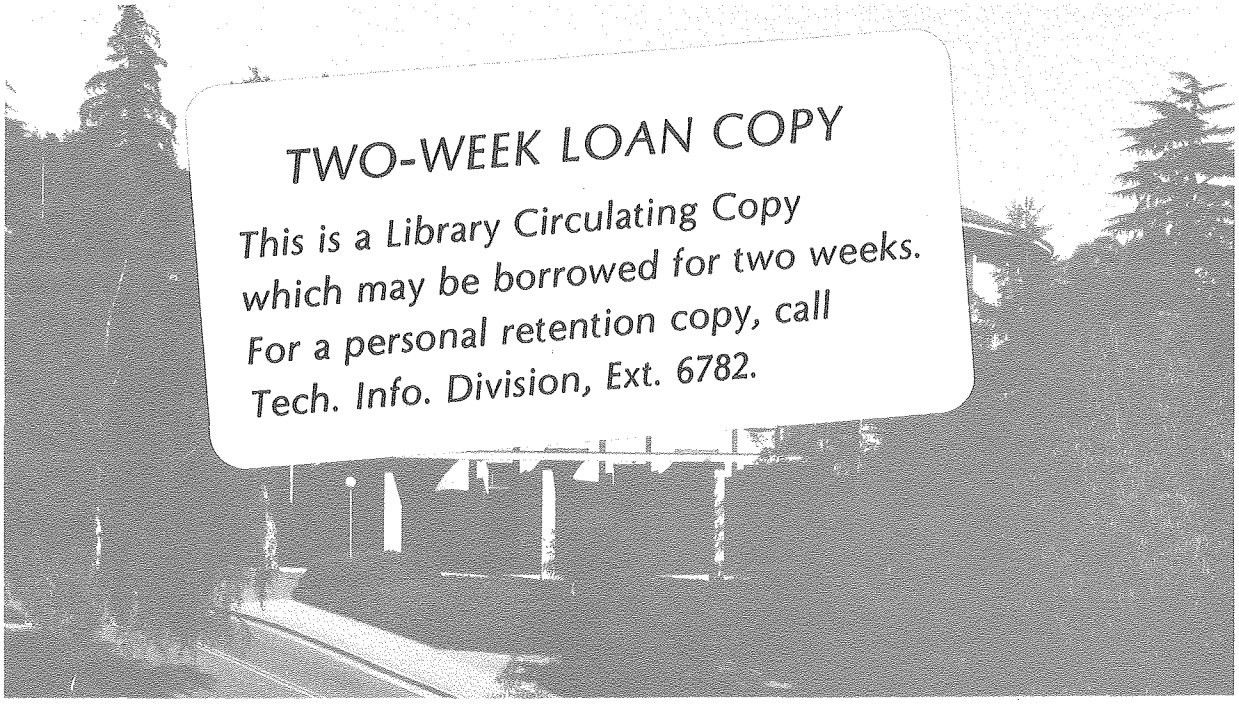

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DISCLAIMER

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INTRODUCTION

The growing of green plants as a renewable energy source is attracting increasing interest (1). The concept of "energy farms" involves the purposeful cultivation of selected species either for use as a solid fuel (wood), or for other energy-rich products. In the latter case, the product is a derivative of the total biomass, and after it is separated from the cellulosic plant material, it can be used directly as diesel fuel in some cases, or it can be converted to a convenient liquid fuel such as gasoline. This approach, the cultivation of plants which already produce hydrocarbon-like compounds is attractive, since the conversion of this type of plant extract to a high quality liquid fuel is expected to be energy efficient, because the material is already in a reduced state.

A large number of plant species are capable of reducing CO_2 beyond carbohydrates to isoprenoids or other hydrocarbon-like compounds. The use of Euphorbias, for example, has been proposed for hydrocarbon production (1). Recently, Buchanan and coworkers have screened several hundred plants for their oil and rubber content (2). Among these Euphorbia lathyris was identified as one of the few potential hydrocarbon-producing crops (3).

Euphorbia lathyris is a herbaceous member of the family Euphorbiaceae. This family of plants consists of approximately 2000 species, ranging from small herbs and succulents to large trees. Perhaps it's best known member is the rubber tree Hevea brasiliensis, whose white, milky latex is the source of natural rubber. Many other Euphorbia species also produce a milky latex which may contain polyisoprenes, and is usually rich in low molecular weight reduced isoprenoids. Euphorbia lathyris is also a latex bearing plant which grows wild in California, consequently we have started to investigate this particular Euphorbia as an "energy farm" candidate.

Fig. 1 shows a close-up view of Euphorbia lathyris. The plants grow with multiple branching in the field to a height of 1.5 m. In the greenhouse, however, Euphorbia lathyris can attain a height of 2.5 m. The best planting time for Euphorbia lathyris in California is early spring, for a late fall (Nov.-Dec.) harvest. In the Southwestern region of the U.S. Euphorbia lathyris can be planted in October and harvested in May or June. For seed production Euphorbia lathyris must be treated as a biennial, i.e. sown in one calendar year and harvested in the next.

The first effort to cultivate this plant started in 1977-78, when test plots from wild seeds were established at the South Coast Field Station of the University of California in Santa Ana. Fig. 2 shows a stand of Euphorbia lathyris in a typical test plot after a 9 month growing season. At this time the plants attained a typical dry weight of 200 g., and grew to a height of approximately 1 m. Planting density was at 1 ft centers and the plots were irrigated receiving a total of 0.5 m of water, 0.25 m of which was natural rainfall. Preliminary results from these experiments indicate that a biomass yield of 22 dry tons hectare^{-1} year^{-1} may be achieved with Euphorbia lathyris. Further agronomic experiments are under way to determine the optimal growing conditions for this plant.

METHODS

Euphorbia lathyris exudes a milky latex when cut. However, this plant is not amenable to continuous tapping like some other Euphorbs. In order to obtain the reduced photosynthetic material, the entire plant is extracted after drying at 70° to 4% moisture content. The reduced organic material is not uniformly distributed throughout the plant; the leaves contain twice as much as the stems per unit weight. Therefore, for uniform sampling, the dried plant is ground in a Wiley Mill to a 2 mm particle size and subsequently thoroughly mixed. A portion of the plant material is then extracted in a soxhlet apparatus with boiling solvent for 8 hrs. Different solvents can be used to extract various plant constituents. One scheme which yields cleanly separated fractions and reproducible results is shown in Fig. 3.

Acetone can also be used as the initial solvent instead of heptane. However, acetone brings down a variable amount of carbohydrates which precipitate out of solution. These can be filtered off, leaving behind a pure acetone soluble portion, which is 8% of the dry weight of the plant. This is equivalent to the sum of Fractions I and II of the extraction scheme shown in Fig. 3.

RESULTS AND DISCUSSION

The heptane extract of Euphorbia lathyris (Fraction I of Fig 3) has a low oxygen content and a heat valve of 42 MJ/kg which is comparable to that of crude oil (44 MJ/kg). These qualities indicate a potential for use as fuel or chemical feedstock material. Therefore we have investigated the chemical composition of this fraction in some detail. Since the amount of the methanol fraction is quite substantial we have also identified the major components of this fraction.

The terpenoids (heptane extract)

The heptane extract is a complex mixture, which can be separated into crude fractions by adsorption chromatography on silica gel. The characteristics of the resulting fractions are shown in Table I. We have examined

each of these column fractions further by gas chromatography and have obtained structural information on the major components by combined gas chromatography-mass spectroscopy (GC-MS). Molecular formulae were obtained by high resolution mass spectroscopy (4).

The data from the GC-MS analyses indicate that over 100 individual components comprise the heptane extract. About 50 of these are major ones; these we have either identified or classified. The major part of the heptane extract consists of various tetra- and pentacyclic triterpenoid functionalized as alcohols, ketones or fatty acid esters. Two representative structures are shown in Fig. 4.

The biosynthetic pathways of terpenoid synthesis in higher plants have been elucidated in some detail, although the regulation of the biosynthetic systems involved is much less effectively documented (5). A common biosynthetic pathway is involved in the early stages of the synthesis of all terpenoids, when the basic five-carbon biological isoprene unit, isopentenyl pyrophosphate (IPP) is synthesized from acetate. An isomerase then converts IPP to dimethylallylpyrophosphate which then initiates the condensations with further molecules of IPP. The continuing pathway leads to all other terpenoids, and is summarized in Fig. 5. The triterpenoids arise via the enzyme mediated cyclization of squalene 1,2-oxide followed by rearrangement sequences to yield a large array of interrelated C_{30} compounds.

In Euphorbia lathyris terpenoid biosynthesis is evidently shunted almost exclusively via this pathway, since no major amounts of any other class of terpenoids have been detected (6). The major terpenoid components of the latex itself have been identified as five triterpenoids (7); all of the latex components with the exception of euphol (a minor one) could also be detected in the whole plant extract. The plant extract, however, yields a much greater variety of triterpenoids than the latex, indicating that terpenoid synthesis must take place in other parts of the plant as well.

The only non-triterpenoid components of the heptane extract are two long chain hydrocarbons, which comprise column fraction I and a small quantity of fatty alcohols isolated from column fraction III. The two hydrocarbons are straight chain waxes: n-$C_{31}H_{64}$ and n-$C_{33}H_{68}$; the three fatty alcohols are $C_{27}H_{53}OH$ $C_{28}H_{57}OH$ and $C_{29}H_{57}OH$. These compounds, however, represent only $\sim$8% of the total heptane extract, so 85% of this extract is composed of only one class of natural products: triterpenoids.

If this Euphorbia lathyris terpenoid extract is to be used as conventional liquid fuel, then further processing of this material is necessary. The conversion of biomass derived hydrocarbon-like materials of high grade transportation fuels has recently been demonstrated by Mobil Research Company (8). Various biomaterials such as triglycerides, polyisoprenes and waxes can be upgraded to gasoline-like mixtures on Mobil's shape selective Zeolite catalyst. The terpenoid extract of

Euphorbia lathyris was processed under similar conditions with this catalyst (9). The product mixture and distribution are shown in Fig. 6.

The products obtained from the conversion of Euphorbia lathyris terpenoids are seen to simulate a gasoline-type mixture; furthermore they are rich in compounds which are premium raw materials for the chemical industry.

The carbohydrates (methanol extract)

As the data in Fig. 3 indicate, a substantial amount (30%) of the dried plant weight can be extracted with methanol. The empirical formula of the water soluble portion of this extract is indicative of carbohydrates.

Since simple hexoses can be directly fermented to ethanol, a useful liquid fuel, we have determined the carbohydrate content of Euphorbia lathyris and identified the specific sugars.

The results of gel-permeation chromatography of Fraction III (Biogel-P-2) indicated that there are no poly- or even oligosaccarides present in this fraction. The carbohydrate containing fractions were identified by the Molish test, and were further characterized by two-dimensional paper chromatography and high pressure liquid chromatography (HPLC). In both of these systems only four simple sugars were detected: sucrose, glucose, galactose and fructose. The HPLC trace of the total sugar fraction as well as the relative amounts of the individual components are shown in Fig. 7.

We have determined that the entire crude carbohydrate (Fraction III, Figure 3) extract is fermentable to ethanol without further purification. Since there is certainly no specific yeast available for Euphorbia sugar fermentation, we have tried several different types: Brewers yeast, Bakers yeast and two yeasts used in the wind industry: Champagne and Montrachet. The best results were obtained with the Montrachet type, a yeast which is very tolerant to phenolic impurities. The fermentations were carried out on 200 ml of an approximately 10% sugar solution to which 130 mg of commercial wine yeasts nutrients were added. The temperature was maintained at 23°C for 90 hrs. Under these conditions an 80% fermentation efficiency was obtained, yielding 8.4 ml of ethanol from 25 gm of the crude water extract. This alcohol yield corresponds to 66%fermentable sugar content of the sample based on the established fermentation efficiency, and is an excellent agreement with the chromatographic data shown in Figure 7. We can therefore obtain not only hydrocarbons from Euphorbia lathyris but a substantial quantity of ethanol as well.

Processing and energy balance

Since Euphorbia lathyris and other hydrocarbon producing crops are new species from the point of view of cultivation, their agronomic characteristics, requirements and yield potentials are not yet well known.

Consequently, any conceptual economic or technical evaluation will contain uncertainities. A recent study by SRI International on the feasibility of growing Euphorbia lathyris for energy use identified the major uncertainities of the feedstock cost and supply (10). This conceptual process study is based on solvent extraction, an existing technology in the seed oil industry. The carbohydrates are recovered by extraction with water. The overall scheme and energy balance are shown in Fig. 8. As seen in Fig. 8 the cellulosic plant residue (bagasse) is used to generate the energy required for solvent extraction and recovery. According to this model, a considerable quantity of bagasse is left over after recovery of the useful products. If an estimate of the required energy input for cultivation is included in this model, the entire process still remains energy positive (11).

Plant selection

In order to determine whether the seed source has any effect on the terpenoid content of Euphorbia lathyris we have first investigated the two cultivars native to California: the Northern and the Southern variety (12). These two ecotypes are shown in Fig. 9. One hundred individual plants of each of these ecotypes were grown in the green house to approximately 100 g fresh weight (4 month growing season). The plant samples were then dried and ground in the usual manner and extracted with refluxing heptane to determine whether there are any differences in terpenoid content between the two seed samples. The frequency distributions for the Northern and the Southern seed source plants are shown in Figures 10 and 11. In both cases the average percent extractables are lower than the 5% obtained from mature field grown Euphorbia lathyris; this is probably due to the shorter growing period as well as the reduced photosynthetic activity in the greenhouse environment. However, as the data indicate the difference between the average percent extractables for the Northern and Southern set is compatible with zero. Northern and Southern yields are within 1% of each other. Therefore there is no statistically significant support for the hypothesis that Northern seed source plants are different from Southern ones in terpenoid content. Furthermore, an assay of the extreme high and low yielding samples of the greenhouse trial (after an additional two month growing period in the field) failed to yield the previously observed high and low groupings. This observation, as well as the low variance within each population, tends to support the conclusion that there is little or no genetically based variance in terpenoid content between these two ecotypes. The observed variance of each set was probably due to different environmental conditions.

Although there are no differences in the quantity of hydrocarbon-like materials produced by these two native California ecotypes, their agronomic characteristics are somewhat dissimilar. In particular, the chilling requirement for flowering is shorter (4 weeks) for the Southern ecotype, then for the Northern one which requires 6 to 8 weeks of chilling at the same temperature (8°C) (13). This property precludes

fall planting of the Southern variety in temperate climates, since flowering will occur at a very small plant size in the following spring. However, in tropical and subtropical regions, where chilling below 10°C does not exceed six weeks, year-round plantings are feasible.

CONCLUSION

Euphorbia lathyris and other potential hydrocarbon-producing crops are new species from the point of view of cultivation. With further agronomic research and plant selection the biomass as well as the terpenoid yield is expected to increase. Nevertheless, it is interesting to compare in terms of energy yield a new crop like Euphorbia lathyris to other established crops such as corn or sugarcane. The liquid fuel yield from corn is 4.2×10^4 MJ $ha^{-1}yr^{-1}$ (14); from sugarcane it is 11.7×10^4 MJ $ha^{-1}yr^{-1}$ (15); both in the form of ethanol. The potential Euphorbia lathyris yield is 6.5×10^4 MJ $ha^{-1}yr^{-1}$ in the form of hydrocarbons and 5.2×10^4 MJ $ha^{-1}yr^{-1}$ in the form of alcohol for a total yield of 11.7 MJ $ha^{-1}yr^{-1}$.

Acknowledgement

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FIGURE AND TABLE CAPTIONS

Figure 1. *Euphorbia lathyris*

Figure 2. Plot of *Euphorbia lathyris*, in Santa Ana, CA

Figure 3. Scheme for extractionof dried *Euphorbia lathyris*

Figure 4. Representative structures for tetra- and pentacyclic triterpenoids found in *Euphorbia lathyris*. The carbon skeletons shows are those of cycloartenol (a) and taraxerol (b).

Figure 5. Pathways of terpenoid biosynthesis

Figure 6. Catalytic conversion of *Euphorbia lathyris* terpenoids

Figure 7. HPLC trace of sugar fraction. Altex NH_2 column. Mobile phase: CH_3CN:H_2O, 80:20

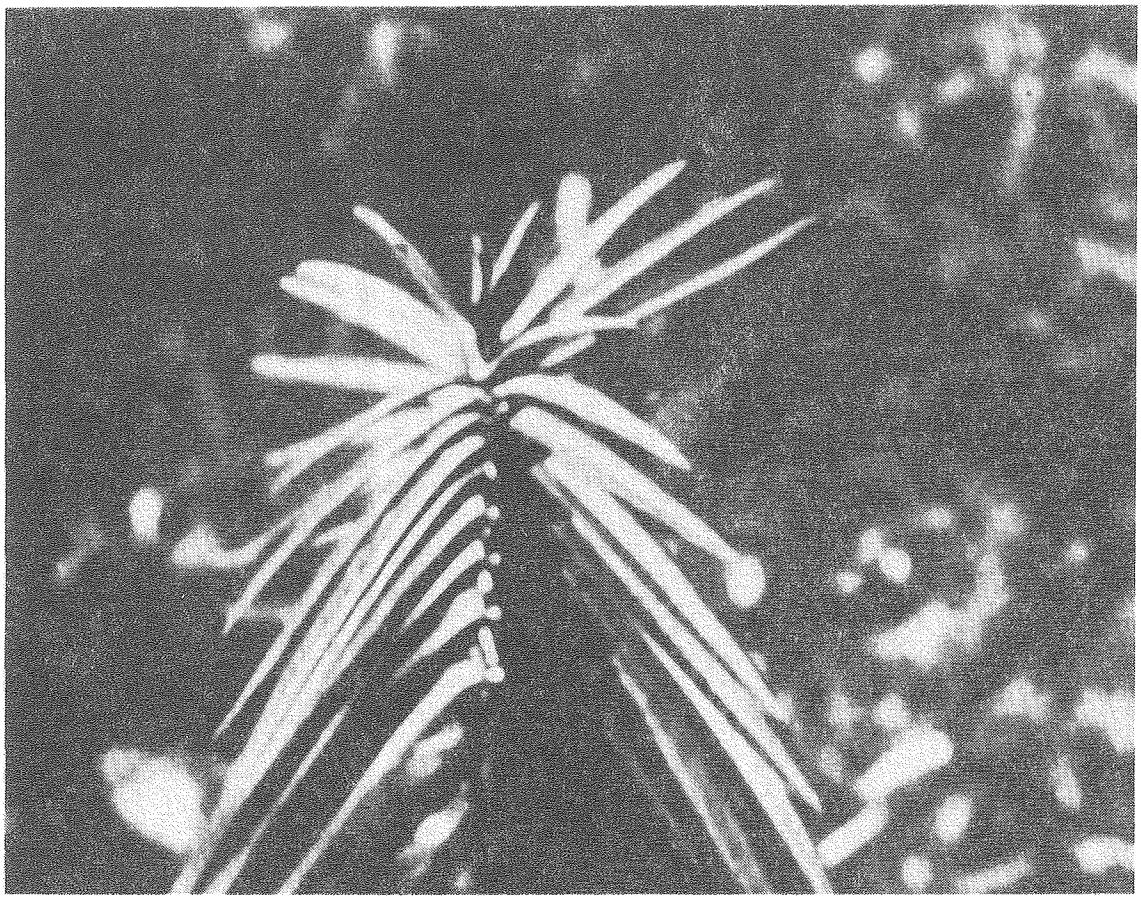
Figure 8. Conceptual processing sequence to recover terpenoids and sugars from *Euphorbia lathyris*. Energy units in MJ are indicated in the parentheses

Figure 9. Northern (on right) and Southern (on left) ecotypes of *Euphorbia lathyris*

Figure 10. Frequency distribution for the Northern ecotype *Euphorbia lathyris*

Figure 11. Frequency distribution for the Southern ecotype *Euphorbia lathyris*

Table I Silica gel column fractions of the heptane extract



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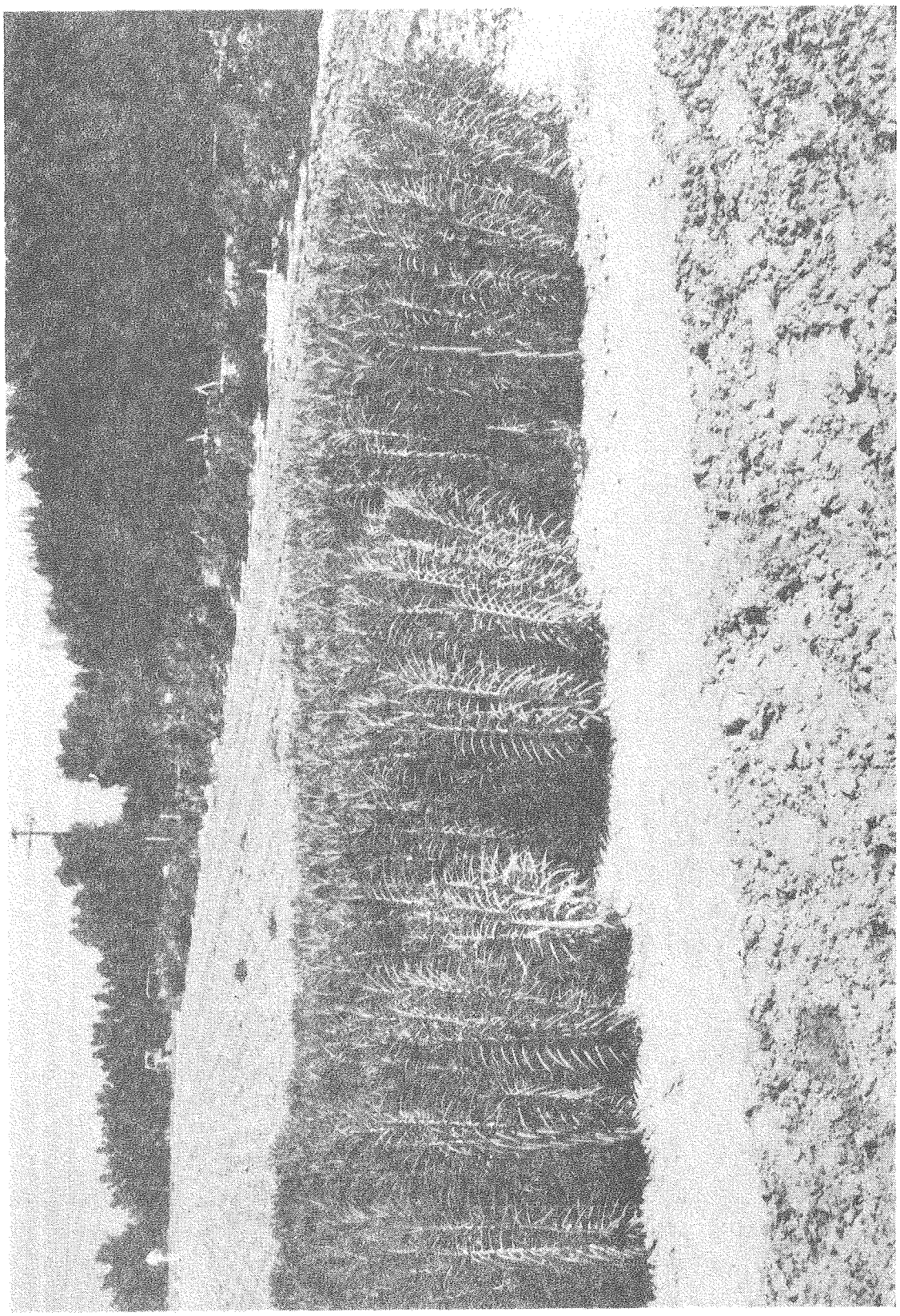

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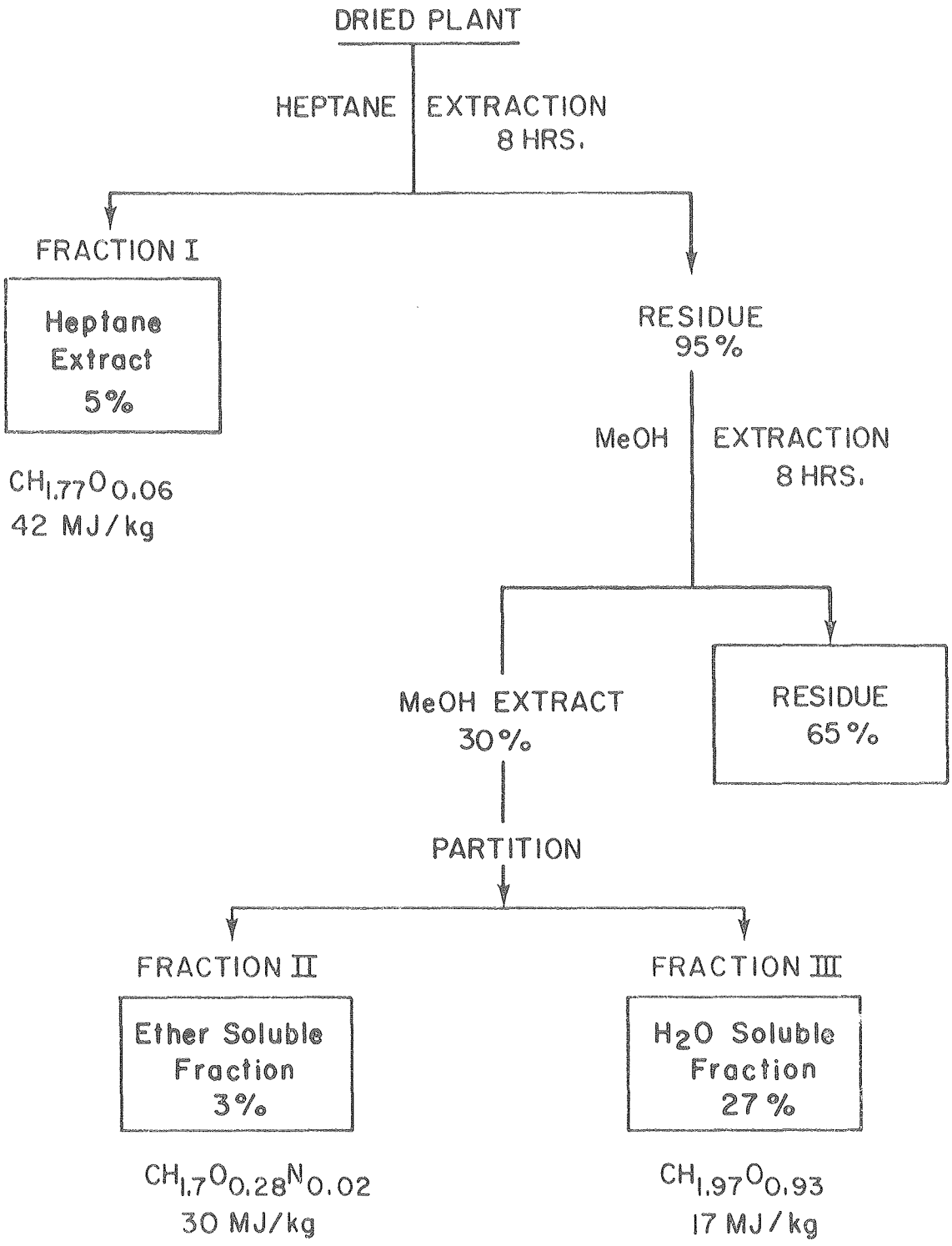
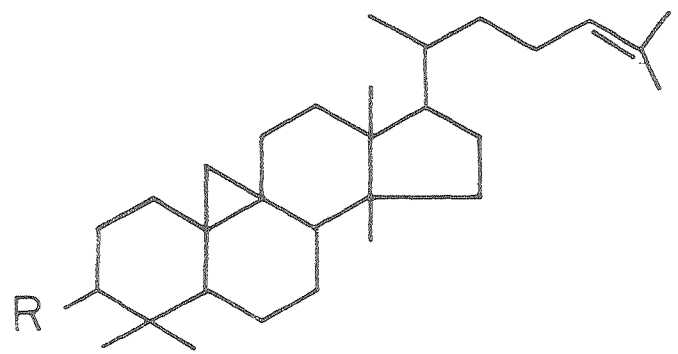


XBL 8010-4417

Eluent		Elemental	(Class of Compounds)
I, heptane	7%	CH_2	Hydrocarbon
II, benzene	33%	$CH_{1.72}O_{0.03}$	Fatty Acid Esters of Triterpenoids.
III, EtoAc	41%	$CH_{1.67}O_{0.07}$	Tetra and Pentacyclic Triterpenoids. Ketones. Alcohols.
IV, Acetone	5%	$CH_{1.63}O_{0.15}$	Phytosterols and Bifunctional Compounds.
V, MeOH	15%	$CH_{1.69}O_{0.22}$	Bifunctional Triterpenoids.

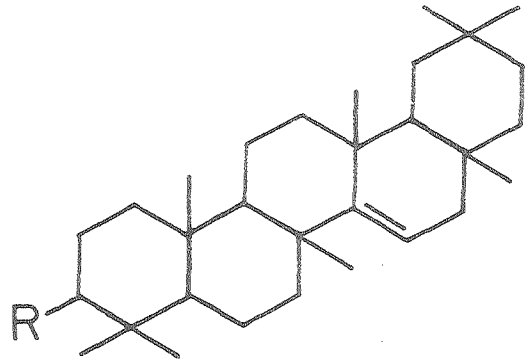
XBL 807-4258

a.



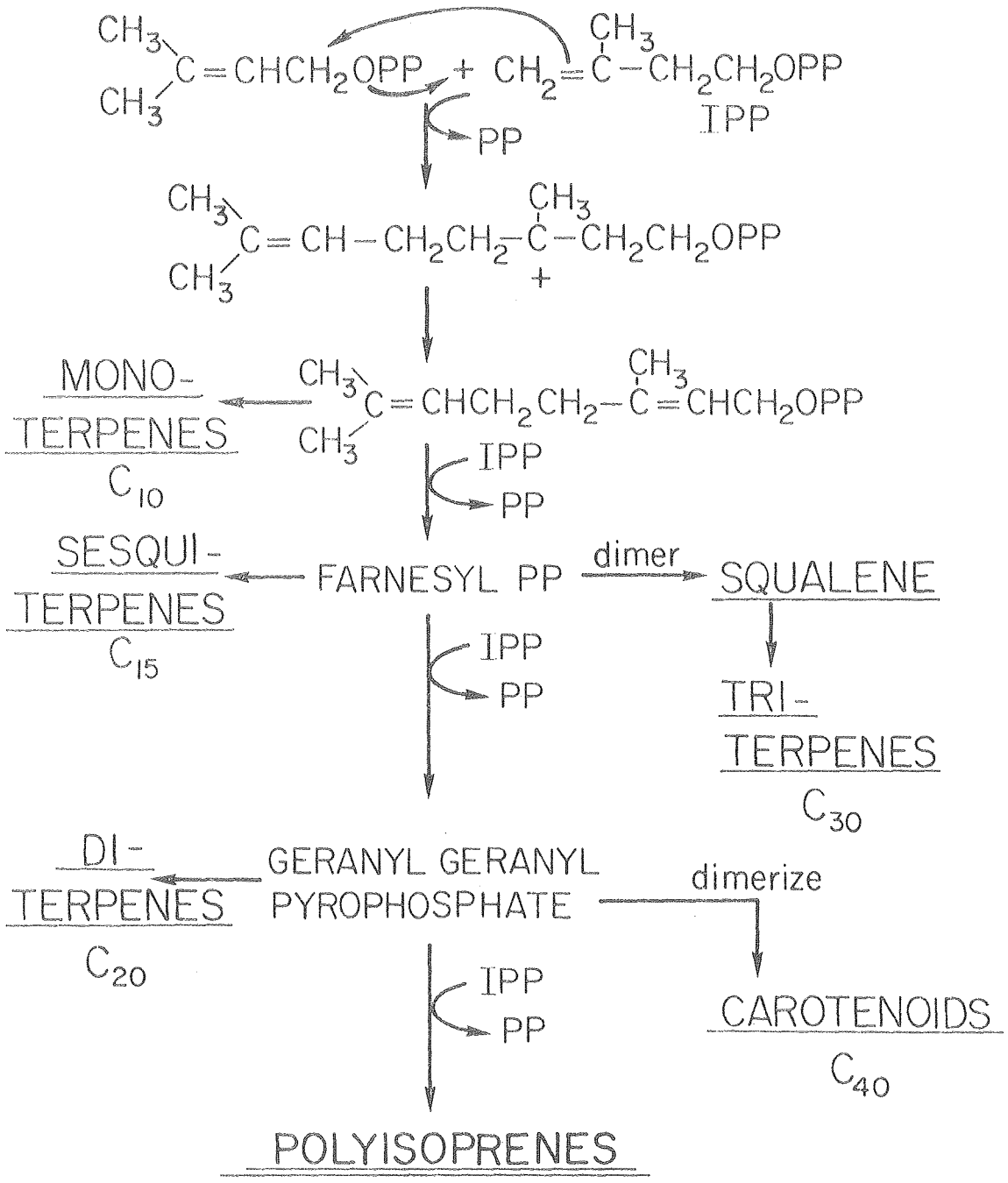
R = OH, or $-O-\overset{\overset{O}{\|}}{C}-(CH_2)_n\,CH_3$

b.



R = OH, or carbonyl

XBL 807-4260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MONO-
TERPENES
C10
IPP
PP
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TERPENES
C15
FARNESYL PP
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TRI-
TERPENES
C30
IPP
PP
DI-
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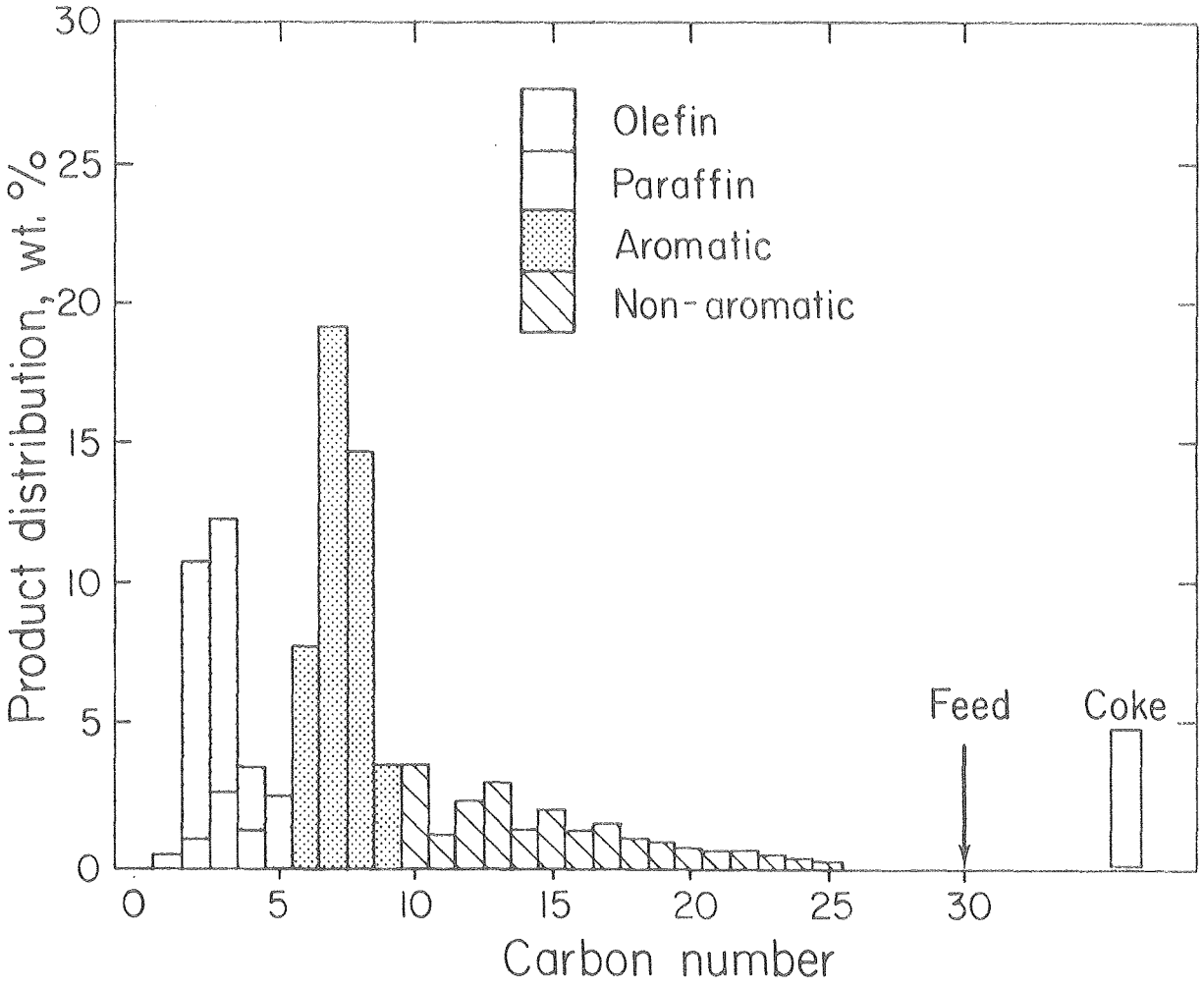
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Feed
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10
15
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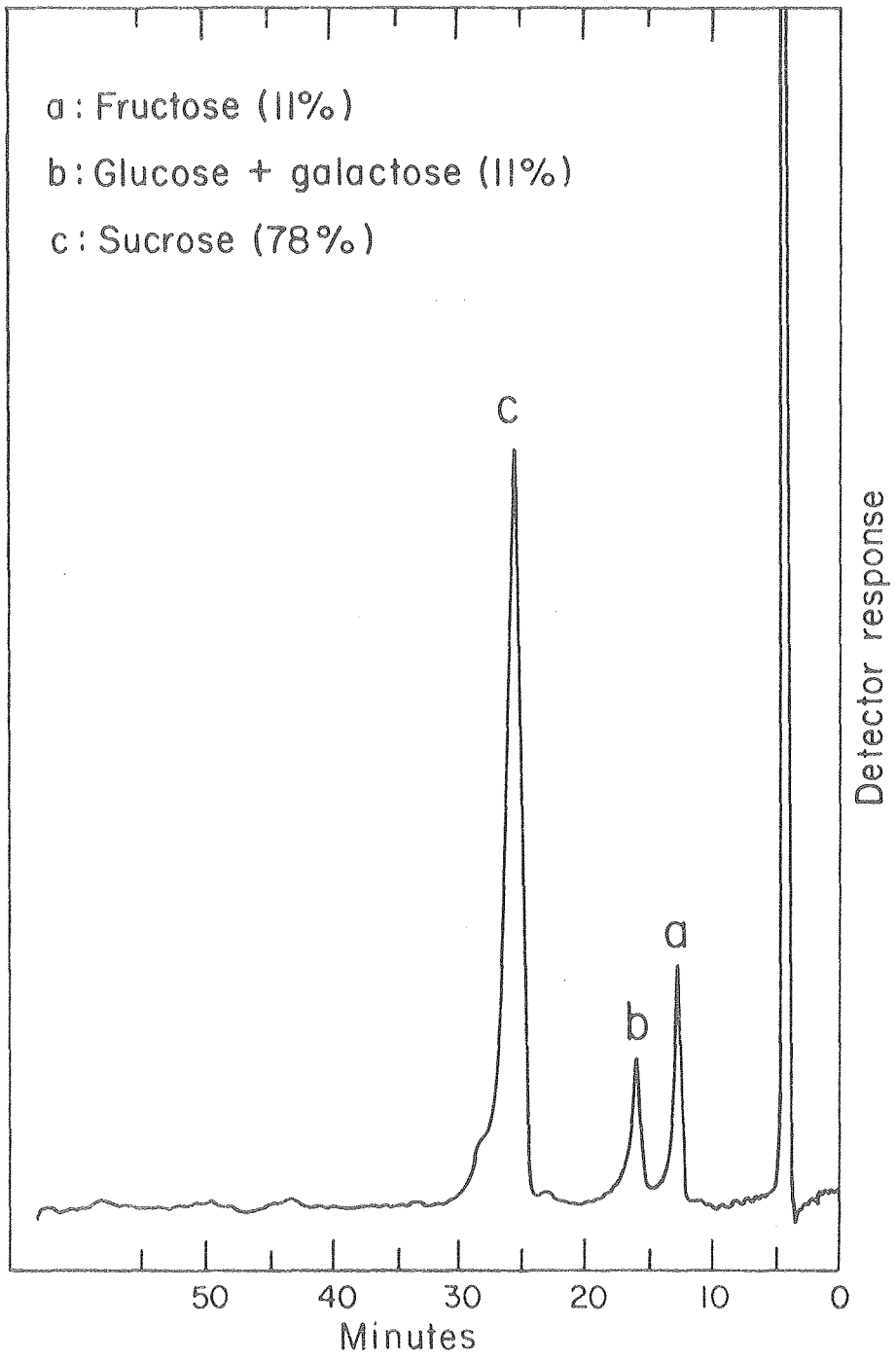
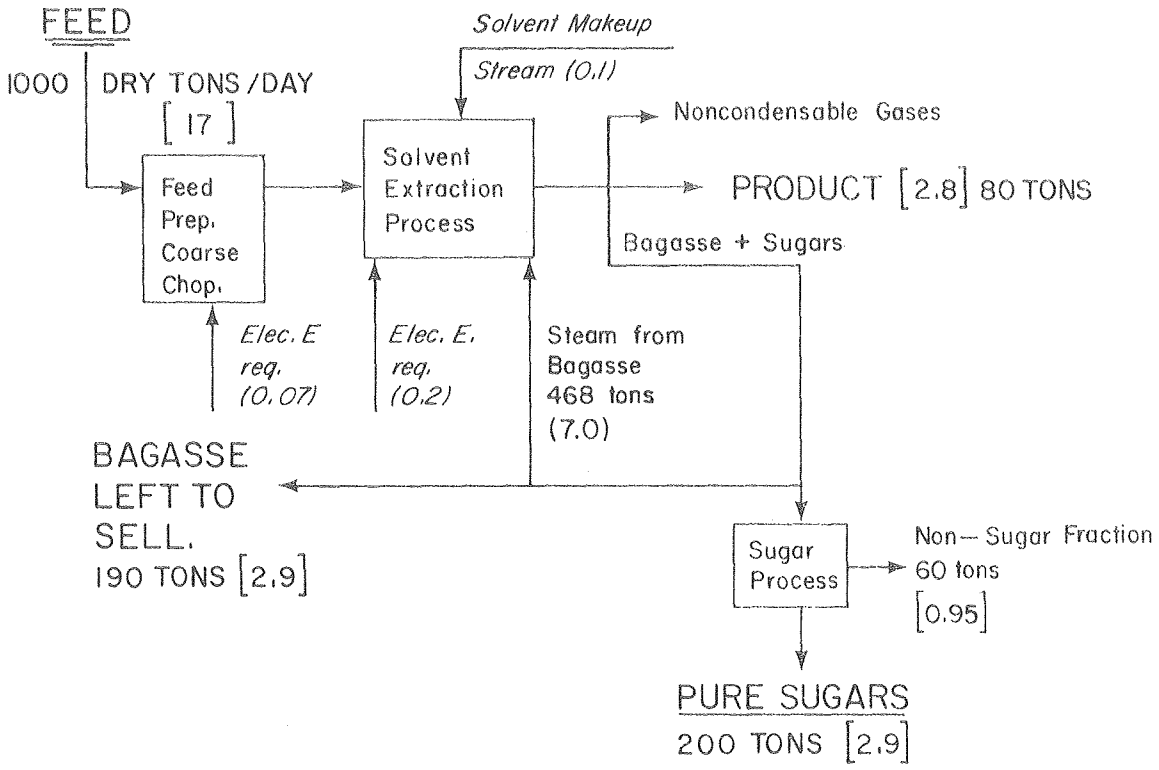
25
30

XBL 807-4261


a : Fructose (11%)
b : Glucose + galactose (11%)
c : Sucrose (78%)
c
b
a
Detector response
50
40
30
20
10
0
Minutes

XBL 807-4264



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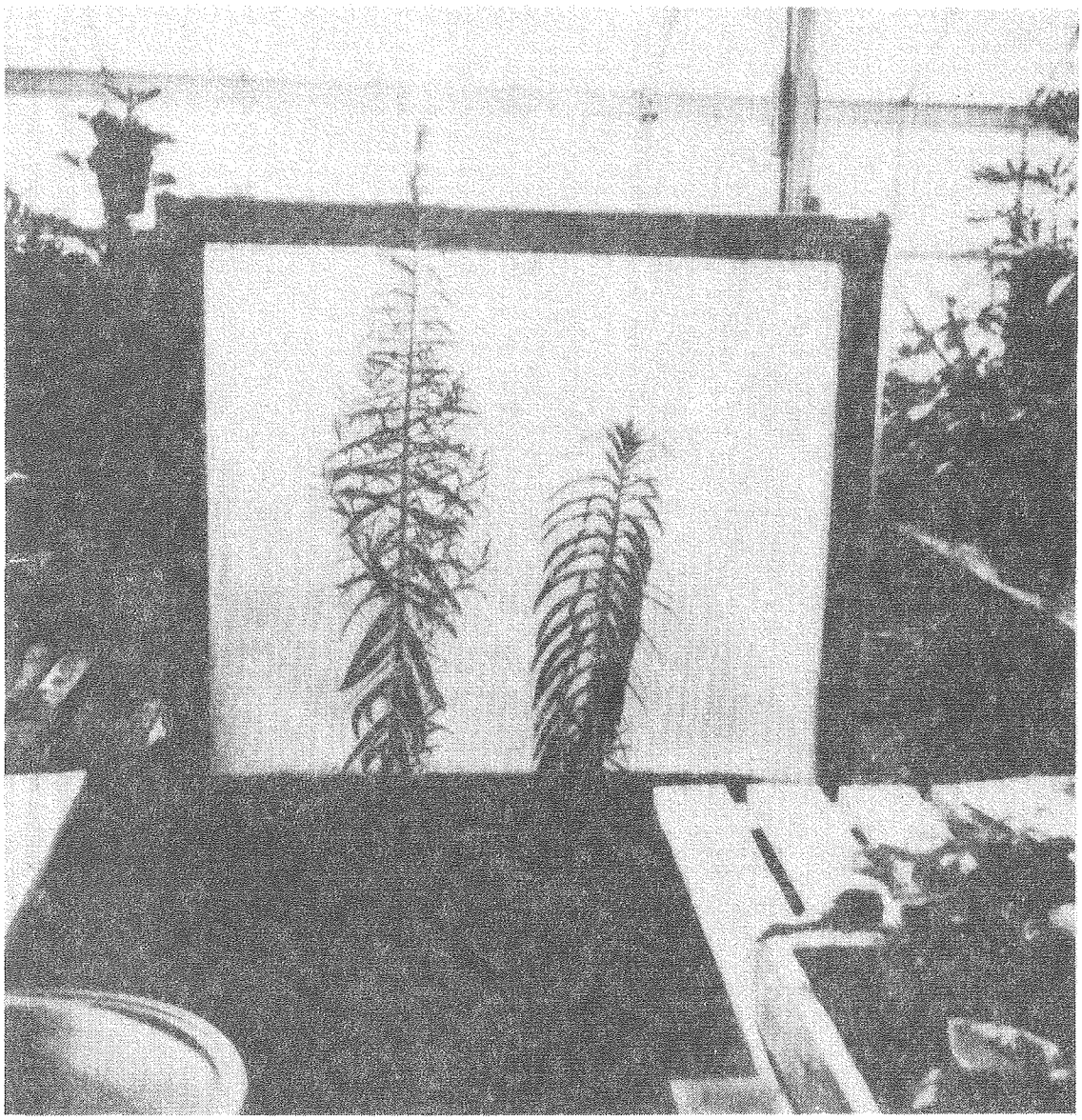

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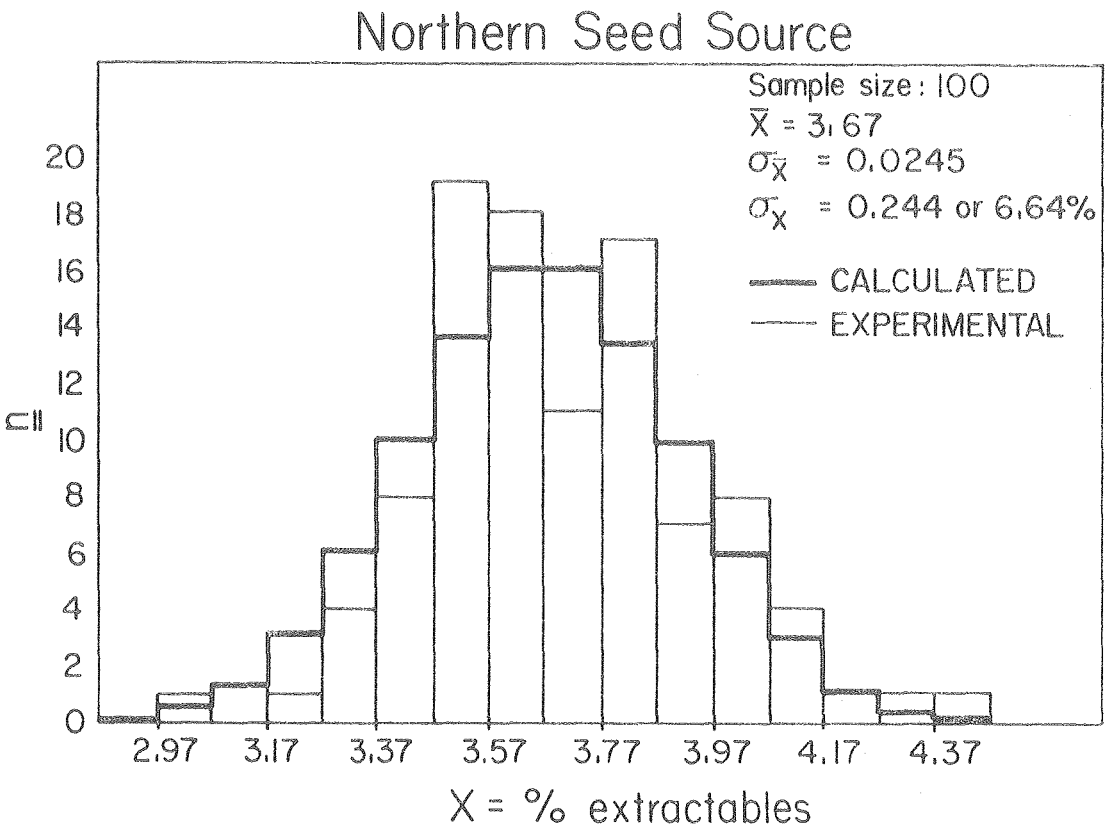
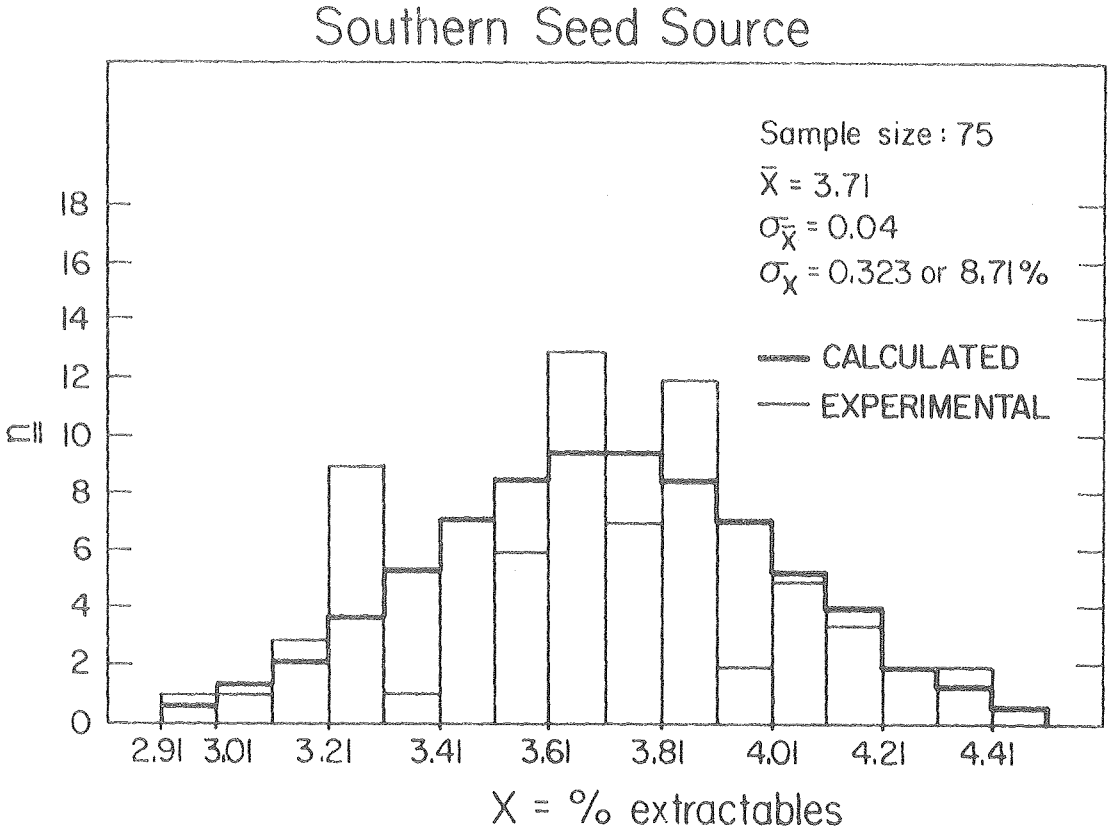

Northern Seed Source
Sample size: 100
$\bar{X}$ = 3.67
$\sigma_{\bar{X}}$ = 0.0245
σ_X = 0.244 or 6.64%
CALCULATED
EXPERIMENTAL
n
0
2
4
6
8
10
12
14
16
18
20
2.97
3.17
3.37
3.57
3.77
3.97
4.17
4.37
X = % extractables

XBL 809-4386


Southern Seed Source
Sample size : 75
$\bar{X}$ = 3.71
$\sigma_{\bar{X}}$ = 0.04
σ_X = 0.323 or 8.71%
CALCULATED
EXPERIMENTAL
n
0
2
4
6
8
10
12
14
16
18
2.91
3.01
3.21
3.41
3.61
3.81
4.01
4.21
4.41
X = % extractables

XBL 809-4387